Medical Progress

Evaluation of the Role of Nitrogen Dioxide in the Development of Respiratory Diseases in Man

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IN RECENT YEARS, considerable evidence has accumulated indicating that nitrogen dioxide is a deleterious component of air pollutants.[1-5] Most of the evidence is descriptive and relates either human or animal exposure to some measurement of pulmonary dysfunction. It is implicit in these experiments that the measured abnormality eventually results in chronic pulmonary disease—for example, emphysema and bronchitis. Since these diseases affect approximately 4 percent of the population and their incidence is increasing,[6] this postulated sequence of events is of paramount practical importance. This report will review the available knowledge concerning the relationship of exposure to nitrogen dioxide and respiratory diseases.

Respiratory Effects of Nitrogen Dioxide In Man and Non-human Animals

The deleterious effects of air pollution are due to the interaction of many pollutants in addition to nitrogen dioxide, and hence a realistic evaluation of the significance of nitrogen dioxide requires data obtained within this context. Unfortunately, the complexities involved in investigating total atmospheres, have, in previous times, been insurmountable. As a result, epidemiological studies have usually contained measurements of only a few of the potential pollutants and experimental models have utilized artificial atmospheres in which nitrogen dioxide was the sole pollutant. It is anticipated that the engineering difficulties which have prevented measuring and simulating ambient atmospheres are near solution and in the future nitrogen dioxide will be studied in a more relevant manner.

Sources of Nitrogen Dioxide Contamination

Nitrogen dioxide enters the atmosphere as a by-product of natural gas combustion, following explosions, in industrial processes requiring the handling of nitric acid, and most importantly from burning petroleum in internal combustion engines. Effluent from gasoline engines contains nitric oxide, a minimally toxic pollutant which at ordinary temperatures is oxidized to the more toxic nitrogen dioxide. Although the complex photochemical reactions governing pollutant interactions are not well understood, sequences describing pollutant interaction have been delineated. According to Haagen-Smit and Wayne,[2] early morning automobile usage produces large quantities of nitric oxide and hydrocarbons. In the presence of sunlight, these products react, converting nitric oxide to nitrogen dioxide so that by mid-morning the atmosphere contains peak

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nitrogen dioxide and low nitric oxide levels. Subsequent irradiation of the nitrogen dioxide produces increasing concentrations of ozone during the afternoon and reduces the nitrogen dioxide to low levels. Late afternoon automobile traffic again produces large amounts of nitric oxide which reacts with the ozone to remove most of this pollutant, and then the nitric oxide continues to reaccumulate at a decreasing rate for the remainder of the night.

As the descriptive sequence indicates, in California the major source of nitrogen dioxide is combustion of automobile fuel; atmospheric concentrations increase in parallel with automobile ownership and use.[3] Thermal power plants are a second, major source of nitrogen dioxide. In heavily industrialized areas these utilities produce 25 to 50 percent of the total oxides of nitrogen.[3]

Although cigarette smoke does not contribute significantly to atmospheric pollution, the high concentrations of nitrogen dioxide which it contains may contribute to the enhanced incidence of respiratory disease of smokers.[7] The pathophysiology of this self-pollution is beyond the scope of this review and will not be considered further.

Information Pertaining to Chronic Exposures in Man

The significance to human health of presently encountered atmospheric levels of nitrogen dioxide is not known.[3,8] The older studies are difficult to evaluate because diagnostic criteria and data collection were inadequate.[9-11] Recent investigations involving healthy populations as well as patients with chronic respiratory diseases have yielded conflicting results. Shy et al compared neighboring communities in Chattanooga exposed to high and low concentrations of nitrogen dioxide.[4,5] They reported a decrease in ventilatory performance and an excess of respiratory illness among families exposed to the increased levels of nitrogen dioxide. Since the source of nitrogen dioxide pollution was a factory producing trinitrotoluene (TNT), other automobile-associated pollutants such as carbon monoxide, hydrocarbons and lead were not considered factors in this study. Spicer and Kerr on the other hand measured pulmonary vital capacity, total lung capacity, functional residual capacity and airway resistance at weekly intervals in 100 seminary students and did not find changes related to atmospheric concentrations of nitrogen dioxide.[12] The effect of exposure to ambient levels of oxidant including nitrogen dioxide on pulmonary function in patients with chronic obstructive respiratory disease has also been investigated.[13-16] In two of these studies pulmonary function was evaluated during alternating periods in which patients breathed Los Angeles smog for a week and then decontaminated air for a week, and in both studies improvement in respiratory function was noted when the patients breathed decontaminated air.[13,14] Objective measurements showing improved ventilatory function were the 3-second timed vital capacity, maximal breathing capacity, and a reduced residual lung volume.[13] It is important to note that particulates and ozone as well as nitrogen dioxide were removed by the purification procedures, and hence these results may not pertain directly to nitrogen dioxide. Rokaw and Massey observed a group of 25 patients with chronic obstructive disease for 18 months and did not find a correlation between air pollution levels including nitrogen dioxide and subjective or objective measurements of pulmonary function.[15] Burrows and coworkers in a larger but less precise investigation also failed to find significant relationships between air pollutant levels and pulmonary function in patients with chronic obstructive pulmonary disease.[16]

Acute Exposures in Man

Definitive evidence demonstrating that exposure to nitrogen dioxide can be deleterious to respiratory function comes from two sources. Acute exposure of humans to high levels of nitrogen dioxide invariably results in respiratory disease.[17-22] In experimental animals exposed to elevated levels of atmospheric nitrogen dioxide pathologic changes resembling emphysema develop and susceptibility to bacterial infection is enhanced.[23-34]

The average level of nitrogen dioxide in atmosphere of smog-ridden areas of California is 0.25 ppm.[2] Air pollution surveys indicate a maximal concentration of 3.5 ppm of nitrogen dioxide.[23] Acute exposure to higher levels of nitrogen dioxide is an uncommon occupational hazard of workers manufacturing nitric acid,[11] of farmers (exposed to silage—silo-fillers' disease),[17,18] and of electric arc workers.[19] A range

of adverse effects correlating with the degree of exposure have been described. Eye and nasal irritation occurs after exposure to 15 ppm of nitrogen dioxide. Pulmonary discomfort is noted at levels of 25 ppm and bronchiolitis with focal pneumonitis occurs after exposures of 25 to 75 ppm of nitrogen dioxide. The duration of these exposures was put at less than one hour. Comparably short periods of exposure to 150-200 ppm causes fatal pulmonary fibrosis. Higher exposures are associated with acute pulmonary edema, sometimes death.[1,17] These studies demonstrate conclusively that elevated concentrations of nitrogen dioxide are extremely toxic to human respiratory tissues.

Volunteer studies further support the hypothesis that acute exposure to concentrations of nitrogen dioxide above ambient, will impair pulmonary function. Abe[20] observed an increased expiratory and inspiratory flow resistance in healthy adults exposed for 10 minutes to 4 to 5 ppm of nitrogen dioxide. Higher levels of exposure (50 ppm) for 1 minute have been shown to cause significant nasal irritation and pulmonary discomfort.[21]

Animal Experiments

The heightened contemporary interest in the potential toxicity of nitrogen dioxide has led to many investigations with experimental animals. These experiments have certain inherent deficiencies. In most cases, the animal model is similar but not completely analogous to the human. As an example, the respiratory anatomy of rats (the animal most frequently used in studies of nitrogen dioxide induced emphysema) differs from that of humans in: not having interlobular septae; having fewer generations of airways; utilizing distal bronchioles for respiration rather than alveoli; pulmonary vasculature.[35] The epithelium of the tracheobronchial tree of rats also differs from man in that it has more large, mucus-secreting glands lining the trachea and fewer along the bronchi.[36] There are also important anatomic differences between humans and rabbits, mice, guinea pigs, monkeys, and dogs.[35] These distinctions in anatomy may explain why identical concentrations of nitrogen dioxide cause diverse pathologic disturbances and make interpretation that is relevant to disease in man exceedingly difficult.

Pathologic Abnormalities Following Exposure to Nitrogen Dioxide

Most studies concerned with the pathology of prolonged exposure to nitrogen dioxide have utilized rodents.[28-30,32,37,38] In rats exposed to 10, 12.5, or 25 ppm of nitrogen dioxide for three or more months the thoracic cavities become larger, dorsal kyphosis develops and the animals have an inflated appearance. Microscopically there is distension of alveolar ducts, dilation of alveoli and hyperplasia of bronchiolar epithelium. Alveolar septa are occasionally missing, but destruction of parenchyma is unusual.[30,37] These pathologic features are similar but not identical with those of human emphysema. A critical difference is the absence of alveolar necrosis. Destructive bullous lesions are the *sine qua non* of emphysema;[39] bullae are absent in rodent models. Also, it should be noted that the lesions mentioned above do not develop in rats exposed to lower concentrations of nitrogen dioxide (0.8 to 2.0 ppm) for their entire lifetime. The lungs from these animals are grossly normal; microscopic examination shows only minor degrees of ciliary loss, epithelial hypertrophy and "cytoplasmic blebbing."[32,38] These animals live out a normal life span and die of diseases unrelated to nitrogen dioxide.[37]

Mice are more susceptible to the toxic effects of nitrogen dioxide than rats. Continuous exposure to 0.5 ppm of nitrogen dioxide for three months causes loss of cilia, alveolar cell disruption and obstruction to respiratory bronchioles.[31] Exposures of longer duration cause more severe changes and pneumonitis.[31] These pathologic abnormalities are, however, unlike the changes of emphysema in man.

Haydon et al exposed rabbits continuously to atmospheres containing 8 to 12 ppm of nitrogen dioxide for 3 to 4 months and reported destructive changes in alveolar walls and abnormal enlargement of the distal air spaces.[27] These findings closely approximate the emphysematous lesions observed in humans. Unfortunately, there are no reports of data obtained from rabbits exposed to lower concentrations of nitrogen dioxide. It should also be noted that other investigators have failed to find emphysematous changes in rabbits exposed for two hours per day to 15 to 25 ppm of nitrogen dioxide for periods up to two years.[40] A multifocal type of emphysema

has been induced in guinea pigs following three weeks of exposure for two hours daily to 22 ppm of nitrogen dioxide.[41] Since these levels are considerably above ambient, the relevance of this model to human disease states is doubtful. The hamster appears to be particularly resistant to the toxic effects of nitrogen dioxide. Kleinerman and Cowdry exposed hamsters to 45 to 55 ppm of nitrogen dioxide for 21 to 23 hours daily for ten weeks and did not find emphysematous changes.[40] The dog is also resistant to the toxic effects of nitrogen dioxide. Wagner et al exposed dogs to 5.0 ppm of nitrogen dioxide for 15 to 18 months and did not find differences between the lungs of treated and control animals.[34] These distinctive results have been confirmed by other investigators.[28,33] Investigations with monkeys are currently in progress but have not yet been reported.[42] Horses, the laboratory animals whose pulmonary anatomy most closely approximates that of man, have, apparently, not been studied.

Effect of Nitrogen Dioxide on Pulmonary Resistance to Infection

Ehrlich and coworkers in a series of experiments have shown that acute and chronic exposure to relatively low levels of nitrogen dioxide depresses pulmonary resistance to infection in mice.[23,24,31] The experimental method consisted of exposing animals to atmospheres of fixed nitrogen dioxide concentration before infection with aerosols of virulent Klebsiella sp. The technique has been sufficiently standardized to predict that 25 to 50 percent of infected controls will die of pneumonia caused by the Klebsiella sp. within 14 days. In a few experiments, bacterial clearance rates were obtained by removing lungs at various time intervals after infection and determining the concentrations of bacteria.

In the acute experiments, mice were exposed for two hours to concentrations of nitrogen dioxide (1.5 to 25 ppm) before infection. Significant increases in mortality occurred in the animals exposed to levels above 3.5 ppm. Deaths did not occur in uninfected mice exposed to identical concentrations of nitrogen dioxide.[23] Further studies with this murine model showed that the adverse effect of nitrogen dioxide is transient. Animals infected with virulent Klebsiella microorganisms 27 hours after exposure to 5, 15 or 25 ppm of nitrogen dioxide did not have an increased mortality when compared with controls.

These investigators also showed that continuous exposures to levels of nitrogen dioxide only slightly above ambient (0.5 ppm or more for three months) depressed murine resistance to pulmonary infection.[24]

Studies of pulmonary bacterial clearance mechanisms using the previously mentioned bacterial clearance technique have demonstrated that the enhancement of murine susceptibility to infection by K. pneumoniae was caused by diminished pulmonary antibacterial activity. Mice which are exposed to nitrogen dioxide and then challenged with aerosols of K. pneumoniae are unable to kill the inhaled bacteria as well as untreated controls. This decrease in bacterial clearance rate is directly proportional to the intensity of exposure to nitrogen dioxide.

Experiments in which hamsters were exposed to nitrogen dioxide and then infected with aerosols of Klebsiella pneumoniae also demonstrated impairment in pulmonary clearance mechanisms, but only at very high concentrations of nitrogen dioxide.[23] This relative increase in resistance to the effect of nitrogen dioxide exposure was attributed to both a diminished virulence of Klebsiella pneumoniae for hamsters and an increased resistance to the adverse effects of nitrogen dioxide.

A few experiments with monkeys have been performed.[25,43] A two hour exposure to 10 to 50 ppm of nitrogen dioxide depressed resistance to aerosol challenge with Klebsiella pneumoniae.[43] Exposure to 10 ppm of nitrogen dioxide for one month or 5.0 ppm for two months also resulted in an enhanced susceptibility to infection. The latter data are preliminary since only a few monkeys were studied; thus, one of four monkeys died following exposure to 10 ppm of nitrogen dioxide and two of seven died at the 5.0 ppm level.[25]

The effect of nitrogen dioxide in combination with other pollutants has been studied by Coffin and coworkers.[44] In these studies, exhaust from an automobile was photochemically treated and conveyed into exposure chambers. Mice within the chambers were exposed to the auto exhaust for four hours and then infected with aerosols of Streptococcus. An enhanced mortality was noted in animals exposed to 25 ppm of carbon monoxide and 0.15 ppm of oxidant. Since nitrogen dioxide forms a significant percentage of exhaust oxidant, it is likely that in this situation it contributed to the adverse effect.

In one report, exposure to nitrogen dioxide did not cause a decrease in pulmonary anti-bacterial activity.[45] Buckley and Loosli exposed germfree and conventional mice to 38 ppm of nitrogen dioxide for six weeks. At the end of this period the animals were infected with aerosols of S. aureus, and bacterial clearance rates were determined during the next five days. Although the rates of bacterial clearance for the germfree and conventional mice differed, neither group was affected by exposure to nitrogen dioxide. The investigators interpreted their data as showing that nitrogen dioxide did not effect bacterial clearance rates. These studies may be criticized on two counts. First, only three animals were studied at each time period—too few for statistical analysis. Second, S. aureus is not pathogenic for mice. Within 24 hours, 99 percent are removed and hence significant differences in clearance that might have occurred within the initial 24-hour period would have been overlooked.

It should be noted that it is very unlikely that the differences in the data of these studies were due to the kinds of microorganisms that were studied (that is, the pathogen K. pneumoniae and the non-pathogen S. aureus). Previous investigations with similar murine models have clearly shown that differences in bacterial virulence cause quantitative but not qualitative changes in clearance rates.[46,47]

Effect of Nitrogen Dioxide on Alveolar Macrophage Function

From the previously cited *in vivo* studies, exposure to nitrogen dioxide appears to inhibit alveolar macrophage function.[23,25,31,43] A few studies have been reported in which macrophages were exposed to nitrogen dioxide *in vitro*.[48,49] According to the data from one investigation, macrophages are killed by exposure to extremely high levels of nitrogen dioxide, 176 ppm.[48] Myrvik and Evans, in a more elegant study, exposed alveolar macrophages from rabbits to 50 ppm of nitrogen dioxide and demonstrated a significant reduction in phagocytic function with a concomitant suppression of cellular energy pathways.[49] Before evaluating data obtained from *in vitro* exposures to nitrogen dioxide, it should be recognized that the effective concentration of nitrogen dioxide in the fluid phase is undoubtedly much lower than in the air phase due to the instability of nitrogen dioxide in water. Hence the atmospheric concentrations used cannot be equated with the levels reported in *in vivo* experiments.[49]

Effect of Nitrogen Dioxide On Mucociliary Function

Few studies relating exposure to nitrogen dioxide to mucociliary function have been performed.[50-52] According to the data obtained, ciliary activity is inhibited by exposure to nitrogen dioxide and this defect results in a decreased rate of particle removal. Certain deficiencies in the laboratory model deserve emphasis. Clearance rates were measured in isolated tracheal segments from either rabbits or rats. An isolated segment is divorced from neuromuscular control, blood supply and the effects of deglutition. Moreover, the rate that particles move along the trachea may not be representative of the rate of function of the entire tracheobronchial tree. Finally, the nitrogen dioxide exposures were short-term and at levels considerably above ambient.

Recently, an improved method of studying mucociliary function has been reported by Spritzer and coworkers.[53] A tight-fitting tube is inserted surgically into the esophagus of the rat and then attached, via the stomach, to an external collecting bottle. Radiolabeled particles are either aerosolized or injected intratracheally and the rate of entrance into the collection bottle is measured. Curves of particle removal for "normal" rats have been reported. Further experiments with this model should allow a more accurate determination of the effect of nitrogen dioxide on mucociliary function.

Effect of Nitrogen Dioxide On Immune Mechanisms

In preliminary studies nitrogen dioxide appears to have an effect upon immune reactions. Matsumura recently demonstrated that exposure of guinea pigs sensitized to egg albumin to 70 ppm of nitrogen dioxide for 30 minutes enhances their susceptibility to systemic anaphylaxis when challenged with aerosols of egg albumin.[54] Exposure to lower concentrations of nitrogen dioxide (40 ppm) causes an increase in the severity of the dyspneic symptoms.[55] Circulating antibody reactive with pulmonary tissue has also been found in guinea pigs that were exposed for as long as

one year to 5.0 to 15.0 ppm of nitrogen dioxide.[56] Since these experiments were conducted at pollutant exposure levels much above ambient, further investigations will be necessary before these data can be related to human instances of pulmonary disease.

Nitrogen Dioxide as a Biological Oxidant

The biochemical mechanisms by which nitrogen dioxide causes cellular dysfunction are in the initial stages of investigation. Since nitrogen dioxide and ozone are similar, it may be that some of the toxic effects of nitrogen dioxide result from biological oxidation to form free radicals.[1] Thomas and associates have presented evidence to support this important hypothesis.[57] These investigators showed an increase in lipoperoxidation of lung lipids in rats exposed to 1.0 ppm of nitrogen dioxide four hours daily for six days. Of practical significance is the additional finding that pre-treatment with high levels of anti-oxidant (10 mg of vitamin E per day) was partially effective in preventing the lipid peroxidative changes induced by nitrogen dioxide. Although it is hazardous to extrapolate from data obtained in laboratory models of infection to instances of human disease, nevertheless these experimental results raise the intriguing possibility that the ingestion of anti-oxidants might prevent some of the deleterious consequences accruing from exposure to nitrogen dioxide.

Conclusion

The studies that have been cited document severe pulmonary disease in individuals exposed acutely to very high concentrations of nitrogen dioxide. However, these concentrations are much above ambient and their relevance to daily environmental exposures is minimal. Since concern about the potential danger of nitrogen dioxide is quite recent, and the postulated disease processes are chronic, definitive information relating pulmonary disease to exposure to nitrogen dioxide under actual, ambient conditions is not available.

There are epidemiological data, however, that support the idea that respiratory impairment may occur in healthy populations and in patients with chronic obstructive respiratory disease following exposure to atmospheric levels of nitrogen dioxide. Although not conclusive, this evidence is sufficient to justify extensive epidemiological investigations designed to determine the significance to human health of exposure to ambient concentrations of nitrogen dioxide. Animal models have served as a valuable means for determining the pathophysiological effects of exposure to pollutants. These studies have shown that exposure to nitrogen dioxide in concentrations that exceed those ordinarily encountered results in pathologic abnormality of the bronchi and alveoli and an enhanced pulmonary susceptibility to bacterial infection.

At present, extrapolation from these data to man is hazardous since animal models do not truly reflect the environmental-host relationships of human exposure. However, as newer techniques are developed, quantitative data delineating the pathophysiological effects, if any, of ambient exposures to nitrogen dioxide should become available and allow insight into the biological consequences of chronic exposure of man to nitrogen dioxide.

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THYROID SUPPRESSION AFTER OPERATION FOR PAPILLARY CANCER OF THE GLAND

Do you use thyroid suppression therapy following operation for papillary cancer of the thyroid gland?

"Yes, I use it for two reasons. The primary reason is the fact that we've done total lobectomy on one side and a subtotal or partial on the other side, leaving 1 to 2 grams of thyroid tissue. This is usually insufficient in amount to prevent myxedema. For this reason, in order to keep the patient euthyroid, I use thyroid replacement therapy.

"Secondly, if by chance there is some truth to the fact that lesions are hormone-dependent, the patient is going to benefit in this respect also."

—OLIVER H. BEAHRS, M.D., Rochester

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